ANNEX 3

EU COMMENTS
ON THE PROPOSED CHANGES TO THE
OIE MANUAL OF DIAGNOSTIC TESTS AND VACCINES
FOR TERRESTRIAL ANIMALS
PRESENTED FOR COMMENTS IN OCTOBER 2021[1]

[1] The draft chapters can be downloaded from the OIE website at the following address: http://web.oie.int/downld/Terr_Manual/MAILING_OCT_2021.zip

EU COMMENTS on the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

SUMMARY

EU COMMENTS on the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

Glossary

General comment:

The EU can in general support the revised glossary. A specific comment is provided below.

Specific comments:

In relation to the proposed definition of "Specific antibody negative (SAN) eggs":

The EU suggests deleting the word "eggs" from the term to be defined. Indeed, the definition refers primarily to animals, while it only mentions "SAN eggs" as an additional use for the term. It thus seems more logical to define "SAN", not "SAN eggs" (or "SAN animals").

1.1.8. Principles of veterinary vaccine production

General comment:

The EU fully supports this revised chapter. The proposed changes are highly appreciated. In line with the cited VICH guidelines, safety tests, such as the laboratory animal toxicity test, are no longer required if a seed-lot system and standard operating procedures are in place. As the VICH guideline 59 is implemented in November 2021, the adaptation of the Terrestrial manual is well in time.

Specific comments:

None

2.3.4. Minimum requirements for the production and quality control of vaccines

General comment:

The EU fully supports this revised chapter. The proposed changes are highly appreciated. In line with the cited VICH guidelines, safety tests, such as the laboratory animal toxicity test, are no longer required if a seed-lot system and standard operating procedures are in place. As the VICH guideline 59 is implemented in November 2021, the adaptation of the Terrestrial manual is well in time.

Specific comments:

None

3.1.4. Brucellosis (infection with *B. abortus*, *B. melitensis*, *B. suis*)

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 75: Please include at the end of the sentence "(e.g., motility)".

LINE 345: Suggest change to "both FM and CITA culture plates per ~~each~~ milk sample tested"

LINE 347: Suggest change to "tryptose broth or trypticase soy broth (TSBA~~A~~)"

LINE 549: Insert space in **TABLE 2**: "Bv. 3: swine".

LINES 691-694: Change to "The ~~highest~~ lowest dilution (in negative goat serum) of this standard that must give a positive result and the ~~lowest~~ highest dilution (in negative goat serum) that must simultaneously give a negative result have been established at 1/16 and 1/200, respectively (McGiven et al., 2011)"

LINE 720: Suggest change to "because of B. abortus S19 vaccination or ~~of~~ FPSR"

LINE 892: Suggest change to "the CFT can be ~~result~~ positive in ruminants after.."

LINES 939-942: Suggest review. The study by Praud et al 2012 on porcine serologic tests does not support the text on Se and Sp of iELISA and RBT for porcine samples.

LINE 998-1001, 1022-1027: The same text is presented in both locations and editing is required. "Several commercial C-ELISAs are available. Some protocols are less sensitive or less specific than others, therefore results obtained from different assays are not always comparable. The cut-off should have been properly established using the appropriate validation techniques (see chapter 1.1.6).

LINE 1269: Suggest change to "Attention must be paid to the conjugate used in the I-ELISA ~~that~~ as it must have a satisfactory…"

LINE 1634: Change to "the spleens can be frozen and kept at ≤ –16°C for ~~from..~~"

LINES 1686, 1888, 2033: Please replace "registration" with "regulatory approval" (same rationale as above).

LINES 717-718: Please replace "Any visible reaction is considered to be positive" with "Any visible colored agglutination is considered to be a positive reaction". Flocculates (false agglutination) are un-interpretable or unreadable. NOTE: Flocculates (easily distinguished from true agglutinates by their aspect) are not rare when testing dog, pig or wild animal sera (Brucellosis EU-RL RBT SOP REV 1, page 4/5, 8 June 2018).

LINE 1750: Suggest change to "For this reason, vaccination of males is ~~counter-indicated~~ contraindicated".

LINES 1304-1305: Regarding brucellin, it is said that an INRA brucellin preparation prepared from a rough strain of *B. melitensis* (CEPA b115) is commercially available. As far as we know it is no longer commercially available, it cannot be purchased, we bought part of the last batch that was manufactured 2 years ago because Zoetis stopped marketing it. We ask you to please check whether it is still commercially available, and if is not, to remove that sentence.

LINES 1770-1772: Suggest a reference be provided to support the sentence "does not always allow the antibody response to RB51 to be distinguished from that induced by field smooth Brucella strains, no matter which S-LPS or OPS-based ELISA is used".

LINES 1886, 2031: Please replace "Requirements for authorisation/ registration/ licensing" with "Requirements for regulatory approval" as that term works globally and has been used in other recently adopted Manual chapters.

LINES 1959-1960: Suggest change to "defective in genes strictly necessary to synthesise perosamine, ~~then~~ and therefore unable to generate OPS antibody response in sheep".

3.1.6. Echinococcosis (infection with *Echinococcus granulosus* and with *E. multilocularis*)

General comment:

The EU thanks the OIE for having taken into consideration comments submitted previously, and can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINES 2-4: Since the intention is to cover zoonotic infections of global public health concern (caused by Eg and Em), it is proposed to further specify in the title the two diseases. The new title would read:

CYSTIC AND ALVEOLAR ECHINOCOCCOSIS (INFECTION WITH ECHINOCOCCUS GRANULOSUS AND WITH ECHINOCOCCUS MULTILOCULARIS)

- *Casulli A. Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis. Lancet Glob Health. 2020 Apr;8(4):e470-e471. doi: 10.1016/S2214-109X(20)30066-8.*

LINE 6: "*Echinococcus granulosus*" is no longer used by the scientific community. It is suggested replacing this wording with ***Echinococcus granulosus sensu lato*** (*E. granulosus s.l.*) - all in italics, according to the international consensus on terminology to be used in the field of echinococcoses. *E. granulosus sensu lato* is a complex of cryptic species.

- *Vuitton DA, McManus DP, Rogan MT, Romig T, Gottstein B, Naidich A, Tuxun T, Wen H, Menezes da Silva A; World Association of Echinococcosis. International consensus on terminology to be used in the field of echinococcoses. Parasite. 2020;27:41. doi: 10.1051/parasite/2020024.*

LINES 9-10: The coproantigen approach was widely used during the 90s', but as now documented in fieldwork, it suffers of drawbacks due to 1) cross-reactions with other Taenidae (affecting specificity in natural populations usually co-infected with other cestodes) and 2) a strong dependency on canid worm burden (affecting sensitivity in low endemic areas). A coproantigen approach is somehow valuable for mass-screening epidemiological evaluations in highly endemic areas (in particular for quantifying the effect of the implemented control programmes), but not appropriate for individual diagnosis. Therefore, it is suggested not quoting "coproantigen" first and to somehow toning down the use of "coproantigen approach" in particular when saying that it is "accurate for diagnosis".

- *Conraths FJ, Deplazes P. Echinococcus multilocularis: Epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. Vet Parasitol. 2015 Oct 30;213(3-4):149-61. doi: 10.1016/j.vetpar.2015.07.027.*

- *Otero-Abad B, Armua-Fernandez MT, Deplazes P, Torgerson PR, Hartnack S. Latent class models for Echinococcus multilocularis diagnosis in foxes in Switzerland in the absence of a gold standard. Parasit Vectors. 2017 Dec 19;10(1):612. doi: 10.1186/s13071-017-2562-1.*

LINE 10: Humans are not intermediate hosts since they do not contribute maintaining the life cycle of these parasites. They are dead-end host for both species of human interest, although as most intermediate animal hosts, they develop metacestode stage. The sentence should therefore be modified accordingly.

- *Casulli A, Siles-Lucas M, Tamarozzi F. Echinococcus granulosus sensu lato. Trends Parasitol. 2019 Aug;35(8):663-664. doi: 10.1016/j.pt.2019.05.006.*

- *Casulli A, Barth TFE, Tamarozzi F. Echinococcus multilocularis. Trends Parasitol. 2019 Sep;35(9):738-739. doi: 10.1016/j.pt.2019.05.005.*

LINES 11-12: It seems that imaging techniques and serology are given the same importance in this sentence. However, serology cannot be used alone. Serology in humans is only "supportive" when imaging technique is inconclusive.

- *Manzano-Román R, Sánchez-Ovejero C, Hernández-González A, Casulli A, Siles-Lucas M. Serological Diagnosis and Follow-Up of Human Cystic Echinococcosis: A New Hope for the Future? Biomed Res Int. 2015;2015:428205. doi: 10.1155/2015/428205.*

LINES 15-23: The list of species in this sentence needs to be reviewed according to the most recent opinions.

- *Thompson RC. Biology and Systematics of Echinococcus. Adv Parasitol. 2017;95:65-109. doi: 10.1016/bs.apar.2016.07.001.*

- *Romig et al. E. multilocularis, E. shiquicus, E. oligarthra, E. vogeli, E. granulosus sensu stricto cluster (G1, G3 and G_{omo}), E. equinus (G4), E. ortleppi (G5), E. canadensis cluster (G6, G7, G8 and G10) and E. felidis. Adv Parasitol. 2017;95, DOI: 10.1016/bs.apar.2016.11.002.*

LINE 19: Use always *E. granulosus sensu lato* (*s.l.*) and *E. granulosus sensu stricto* (*s.s.*) in italics according to the above cited consensus on Echinococcosis.

LINE 19: "G1,3 and other G" are genotypes (not strains). The wording should be modified accordingly.

LINE 26: It is better to use *E. granulosus s.l.* in order to consider the complex species as a whole.

LINES 40-41: It is important to stress that coproantigen tests are not the main technique used for Em surveillance in the EU. Surveillance of Em is performed by collection of samples from wild definitive hosts using the sedimentation and counting technique (SCT) on intestinal contents or using PCR DNA techniques for the detection of the parasite in faecal samples.

- *Conraths FJ, Deplazes P. Echinococcus multilocularis: Epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. Vet Parasitol. 2015 Oct 30;213(3-4):149-61. doi: 10.1016/j.vetpar.2015.07.027.*

LINES 62-73: Please harmonise the taxonomy in accordance with the most recent literature (see comment to lines 15-23).

LINE 73: Please replace “Strains G6 and G7” by “Genotypes G6/7” as they are not strains but genotypes and considered as a cluster of genotypes.

- *Addy F, Wassermann M, Kagendo D, Ebi D, Zeyhle E, Elmahdi IE, Umhang G, Casulli A, Harandi MF, Aschenborn O, Kern P, Mackenstedt U, Romig T. Genetic differentiation of the G6/7 cluster of Echinococcus canadensis based on mitochondrial marker genes. Int J Parasitol. 2017 Dec;47(14):923-931. doi: 10.1016/j.ijpara.2017.06.003.*

LINE 75-76: Please note that *E. shiquicus* has only been found in the Tibetan plateau (small part of China) and *E. oligarthra* only documented in North America (only in Mexico).

LINE 77: Using “Forms” sounds like referring to a same disease, whereas these are totally different diseases (epidemiologically and clinically). It is proposed to refer to “causing various echinococcosis diseases”.

LINE 77-78: There is now scientific evidence that *E. equinus* is zoonotic and infecting people in Turkey and Uzbekistan (see below). It should therefore be removed from the sentence.

- *Macin S, Orsten S, Samadzade R, Colak B, Cebeci H, Fındık D. Human and animal cystic echinococcosis in Konya, Turkey: molecular identification and the first report of E. equinus from human host in Turkey. Parasitol Res. 2021 Feb;120(2):563-568. doi: 10.1007/s00436-021-07050-w.*

- *Kim HJ, Yong TS, Shin MH, Lee KJ, Park GM, Suvonkulov U, Kovalenko D, Yu HS. Phylogenetic Characteristics of Echinococcus granulosus Sensu Lato in Uzbekistan. Korean J Parasitol. 2020 Apr;58(2):205-210. doi: 10.3347/kjp.2020.58.2.205.*

TABLE 1: *E. granulosus s.l.* is cosmopolitan, with the exception of Antarctica.

LINES 93-94: It is difficult to speak of a true specificity for the *Echinococcus* species that are quoted. It is more a predilection in the sense that the life cycle of these

Echinococcus species is maintained mainly by some intermediate animal host species but parasites can infect a really wide range of intermediate hosts (therefore, no specificity).

LINE 101: According to the international consensus on terminology (see below), the term “hydatids” should be discouraged and replaced by “larval stage” or “metacestode” or “echinococcal cyst”.

- *Vuitton DA, McManus DP, Rogan MT, Romig T, Gottstein B, Naidich A, Tuxun T, Wen H, Menezes da Silva A; World Association of Echinococcosis. International consensus on terminology to be used in the field of echinococcoses. Parasite. 2020;27:41. doi: 10.1051/parasite/2020024.*

LINE 150: According to the international consensus on terminology (see below), the wording “polycystic echinococcosis” is not appropriate since it usually presents as a single cyst. It is proposed to refer for *E. oligarthra* and *E. vogeli* to neotropical echinococcosis.

- *Vuitton DA, McManus DP, Rogan MT, Romig T, Gottstein B, Naidich A, Tuxun T, Wen H, Menezes da Silva A; World Association of Echinococcosis. International consensus on terminology to be used in the field of echinococcoses. Parasite. 2020;27:41. doi: 10.1051/parasite/2020024.*

LINE 171: Please note that this sentence on meat inspection only refers to species causing cystic echinococcosis, but not alveolar echinococcosis where microrodents are the most relevant intermediate host species.

LINE 220: It is proposed to replace “AND sequencing” by “AND WHERE NEEDED sequencing”, since there are some recent detection methods specifically developed to identify most species/genotypes and avoid sequencing.

- *Santolamazza F, Santoro A, Possenti A, Cacciò SM, Casulli A. A validated method to identify Echinococcus granulosus sensu lato at species level. Infect Genet Evol. 2020 Nov;85:104575. doi: 10.1016/j.meegid.2020.104575.*

- *Maksimov P, Bergmann H, Wassermann M, Romig T, Gottstein B, Casulli A, Conraths FJ. Species Detection within the Echinococcus granulosus sensu lato Complex by Novel Probe-Based Real-Time PCRs. Pathogens. 2020 Sep 26;9(10):791. doi: 10.3390/pathogens9100791.*

LINES 238-244: “The small intestine is removed as soon as possible after death, and tied at both ends. If the material is not frozen or formalin fixed (4–10%), it should be examined quickly, as the parasite can be digested within 24 hours. [...] The fresh intestine is divided into several sections and immersed in 0.9% saline at 38±1°C for examination [...]”. Indeed, laboratory personnel carrying out these procedures are exposed to the risk of infection and severe disease, which must be minimised by appropriate biosafety and containment procedures. Any examination of the intestines

of potential definitive hosts without prior freezing is a very dangerous procedure. Infective (egg/adult) material can be decontaminated by freezing at –80°C (core temperature) for 5 days. We therefore suggest to insert a reference to the importance of carrying out the freezing procedure in **LINES 238-244**, as it is only mentioned a bit later, in **LINES 254-256**.

LINE 256: It may also be added that metacestode of *E. multilocularis* can survive in liquid nitrogen (around -200°C) for 35 years and be infective again.

- *Laurimäe T, Kronenberg PA, Alvarez Rojas CA, Ramp TW, Eckert J, Deplazes P. Long-term (35 years) cryopreservation of Echinococcus multilocularis metacestodes. Parasitology. 2020 Aug;147(9):1048-1054. doi: 10.1017/S003118202000075X.*

LINE 259: It may be worth to refer also to the segmental sedimentation and counting technique (SSCT), which can be used with slightly similar sensitivity but a less time-consuming nature.

- *Umhang G, Woronoff-Rhen N, Combes B, Boué F. Segmental sedimentation and counting technique (SSCT): an adaptable method for qualitative diagnosis of Echinococcus multilocularis in fox intestines. Exp Parasitol. 2011 May;128(1):57-60. doi: 10.1016/j.exppara.2011.01.004.*

LINE 310: It is suggested toning down the wording and replace “offers” by “may offer” as a better alternative to necropsy is DNA-based methodologies in faeces (see comments on coproantigen assays to lines 9-10).

LINES 372-383, TABLE 3: It is suggested to review these parts and use the information contained in the more recent publications focusing on detection:

- *Siles-Lucas M, Casulli A, Conraths FJ, Müller N. Laboratory Diagnosis of Echinococcus spp. in Human Patients and Infected Animals. Adv Parasitol. 2017;96:159-257. doi: 10.1016/bs.apar.2016.09.003.*

Moreover, some more recent protocols can be implemented in the context of *Echinococcus* spp. detection, as follows:

- A 2 step approach (PCR-RFLP and multiplex PCR) to detect, avoiding sequencing, all the species belonging to *E. granulosus s.l.:*

  Santolamazza F, Santoro A, Possenti A, Cacciò SM, Casulli A. A validated method to identify Echinococcus granulosus sensu lato at species level. Infect Genet Evol. 2020 Nov;85:104575. doi: 10.1016/j.meegid.2020.104575.

- A real time PCR using melting curves to detect all the species belonging to *E. granulosus s.l.:*

Maksimov P, Bergmann H, Wassermann M, Romig T, Gottstein B, Casulli A, Conraths FJ. Species Detection within the Echinococcus granulosus sensu lato Complex by Novel Probe-Based Real-Time PCRs. Pathogens. 2020 Sep 26;9(10):791. doi: 10.3390/pathogens9100791.

- A sensitive real time PCR for coprodiagnosis of *E. multilocularis*:

  Knapp J, Umhang G, Poulle ML, Millon L. Development of a Real-Time PCR for a Sensitive One-Step Coprodiagnosis Allowing both the Identification of Carnivore Feces and the Detection of Toxocara spp. and Echinococcus multilocularis. Appl Environ Microbiol. 2016 May 2;82(10):2950-2958. doi: 10.1128/AEM.03467-15.

LINE 422: It is suggested to replace “reduce transmission of cystic echinococcosis to humans” by “reduce human exposure to cystic echinococcosis”, as this is the parasite which is transmitted but not the disease (i.e. cystic echinococcosis).

LINE 503: Please replace “registration dossier” with “<u>regulatory approval</u> dossier”, for consistency with wording in LINES 54 and 433.

3.1.8. Foot and mouth disease (infection with foot and mouth disease virus)

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 5: Please insert the word "highly" before "contagious disease" (consistent with text in **LINE 29**).

LINE 26: It should be "Detection of the agent", not "Identification of the agent", for consistency with **TABLE 1** and **LINE 57**.

LINE 46: The first subheading in **TABLE 1** should not be "Detection of the identification", but "Detection of the agent".

Furthermore, the footnote (b) should be placed with "Virus isolation" in the first column, not the "+++" in the column under "Confirmation of clinical cases". Indeed, that footnote is relevant for all test purposes.

Finally, footnote (a) and (b) should be inverted, as the new footnote (b) comes first in the table (i.e. footnote (a) to become (b) and vice versa).

LINE 52: This should be "RT-PCR = reverse-transcription polymerase chain reaction" instead of "RT-PCR = reverse-transcriptase polymerase chain reaction".

LINE 60: Instead of "ELISA CF and the lateral flow device", this should be "ELISA, CF and LFD".

LINE 65: "Vaccine-induced" should be hyphenated.

3.1.14. Nipah and Hendra virus diseases

General comment:

The EU can in general support this revised chapter.

However, the summary and introduction sections should be revised for better readability, as the text has been modified multiple times. This also applies (to a lesser extent) to the methods section.

Indeed, the introduction is jumping between Nipah Virus, Hendra Virus and African Henipavirus. It would thus be good to re-organize and also to add current information about the detection range of African Henipaviruses.

As NRL and OIE Collaborating Centre for Zoonoses in Europe, Germany's Friedrich-Loeffler-Institute (FLI) offers its willingness to participate in this process.

Specific comments are provided below.

Specific comments:

LINE 88: Replace "registered" with "approved" (agreed international terminology used in other recently adopted chapters of the Manual).

LINE 167, Table 1: Notes 1 and 2 are missing in the legend. The symbols in the first line (virus isolation) are not readable.

LINE 251: Please add "or paraformaldehyde" after "aceton" as also paraformaldehyde can be used for fixation.

LINE 483f: Details are missing; the type of ELISA plate and the protein concentration used need to be specified.

LINE 518f: This limitation can be overcome by using a different conjugate.

LINE 526: Details are missing; the type of ELISA plate and the protein concentration used need to be specified.

LINE 562ff: Other ELISA protocols for henipavirus diagnostics in pigs have been published,
e.g. Fischer K, Diederich S, Smith G, Reiche S, Pinho dos Reis V, Stroh E, *et al*. (2018) Indirect ELISA based on Hendra and Nipah virus proteins for the detection of henipavirus specific antibodies in pigs. PLoS ONE 13(4): e0194385. https://doi.org/10.1371/journal.pone.0194385

LINE 580: Details are missing; the type of ELISA plate need to be specified.

LINE 586f: "…to each well as indicated in the format below. Add 100 µl…":
the method refers to a format given below, but this is missing.

LINES 626-629: The statement in the last sentence is no longer current, since all ELISA protocols are now established with recombinant antigen which is not associated with any biosafety risk.

3.1.22. Tularemia

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 13: Please add: "Oropharyngeal and pneumonic infections can be caused by ingestion of contaminated food and water or inhalation of aerosols, respectively." Indeed, in combination with the comments of others in the next paragraph this is relevant with regard to work-safety issues in the lab and the potential use of F. tularensis as a biological agent. Animals may be indicators of an outbreak and veterinarians may be first responders.

LINE 16: ~~It is important to understand that there is a~~ recognise the ~~high risk of direct infection of humans by~~ via aerosols and ~~direct contact with the organism~~ or infected ~~tissue.~~ The disease spreads through vectors such as mosquitoes, horseflies, deer flies, and ticks. Humans have a high risk of acquire the disease through direct contact of sick animals, infected tissues, consumption of infected animals, drinking or direct contact of contaminated water, and inhalation of bacteria-loaded aerosols.

LINE 54: infected, and the organism has been detected isolated from birds, fishes, crayfish, amphibians, arthropods, and protozoa (Gyuranecz, 2012; Morner & Addison, 2001; Yeni et al., 2020, Anda et al 2001).

LINE 90: Please delete the words "and faeces", or provide references that prove that living bacteria occur in faeces of mammals.

LINE 91: Infection can occur by simple contact of sick animals, infected tissues, consumption of infected animals, drinking or direct contact of contaminated water and via inhalation of infective aerosols.

TABLE 1: Real-time PCR / Population freedom from infection should be "+". Indeed, this is only useful in very limited circumstances (e.g. in ticks or other arthropods), but has little or no relevance for screening asymptomatic animals.

TABLE 1: Conventional PCR / confirmation of clinical cases should be "+++". Indeed, PCR is a reliable tool for confirmation of clinical cases as pointed out in Chapter 1 **LINES 113/114**.

LINE 129: Suggest change to "fluorescein isothiocyanate".

LINE 160: Cystine heart agar broth with blood (CHAB).

LINE 160: Change to "specimens" (typo).

LINES 187-188: Usually 30 amplification cycles are applied for strains analyses, but for samples, where *Francisella* may be in low concentration, it's better 40 cycles of amplification.

LINE 201: Please add the following 2 references:

Broekhuijsen, M. et al. 2003: Genome-Wide DNA Microarray Analysis of Francisella tularensis Strains Demonstrates Extensive Genetic Conservation within the Species

but identifies Regions that are Unique to the Highly Virulent F. tularensis subsp. tularensis. J. Clin. Microbiol. 41(7), 2924-2931.

Tomaso H, Scholz HC, Neubauer H, Al Dahouk S, Seibold E, Landt O, Forsman M, Splettstoesser WD. Real-time PCR using hybridization probes for the rapid and specific identification of Francisella tularensis subspecies tularensis. Mol Cell Probes. 2007 Feb;21(1):12-6.

The methods described in these papers have been successfully used in ring-trials in accordance with ISO 17025.

LINE 223: An alternative method for the inactivation of *Francisella tularensis* is suggested because, according to our experience, bacterial inactivation may not be complete with the use of alcohol and without keeping the suspension under continuous stirring. However, it is always advisable to verify the complete inactivation of the bacterial suspension before use. See also comment line 186-187

LINE 273: Anda, P., Del Pozo, J. S., García, J. D., Escudero, R., Peña, F. G., Velasco, M. L., Sellek R.E., Jiménez M.R., Sánchez L.P & Navarro, J. M. (2001). Waterborne outbreak of tularemia associated with crayfish fishing. *Emerging infectious diseases*, *7*(3 Suppl), 575.

Please add a section on the identification of *Francisella tularensis* using MALDI-TOF MS, as follows:

"**3.1 Identification using MALDI-TOF MS**

F. tularensis species can be identified with MALDI-TOF MS if corresponding reference spectra are available in the database. The preparation is carried out using the ethanol extraction method (according e.g. to the Bruker Daltonics protocol). After 20 minutes in ethanol, the bacteria are killed and the usual preparation can be carried out safely. Picking and direct application of the colonies to the target is not recommended (suggestions for references below the Literature section)."

López-Ramos I, Hernández M, Rodríguez-Lázaro D, Gutiérrez MP, Zarzosa P, Orduña A, March GA. Quick identification and epidemiological characterization of Francisella tularensis by MALDI-TOF mass spectrometry. J Microbiol Methods. 2020 Oct;177:106055.

Regoui S, Hennebique A, Girard T, Boisset S, Caspar Y, Maurin M. Optimized MALDI TOF Mass Spectrometry Identification of Francisella tularensis Subsp. holarctica. Microorganisms. 2020 Jul 28;8(8):1143.

Seibold E, Maier T, Kostrzewa M, Zeman E, Splettstoesser W. Identification of Francisella tularensis by whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry: fast, reliable, robust, and cost-effective differentiation on species and subspecies levels. J Clin Microbiol. 2010 Apr;48(4):1061-9.

MALDI-TOF MS is a very quick and reliable method for the identification of these bacteria as was demonstrated in the literature.

Finally, please add a section on molecular epidemiology for outbreak investigations, as follows:

"**4.1. Molecular epidemiology**

Outbreak investigations are of high importance especially in zoonotic bacteria that are also classified as potential biological agents such as *F. tularensis*. Real-time PCR assays targeting insertion/deletion-loci or canonical SNPs allow assignment of strains to genetic clusters with high discriminatory power (Svensson *et al.*, 2009). In recent years, whole genome sequencing (WGS) has been used to elucidate the evolution and spread of the bacterium, but also to investigate outbreaks (References). Ring trials of international organisations (e.g. UNSGM) already include this technology as a standard procedure for the characterization of isolates. Bioinformatics pipelines are freely available: open-source canSNPer software (canSNPer; https://github.com/adrlar/CanSNPer).

NGS data analysis starts with a quality control. In this process, 70% of the bases of a sample should have a quality score (Phred score) of at least 30 (corresponds to an error probability of 0.001). Quantitatively, a coverage of at least 30 should be achieved, whereby at least 70% of the reads should be taxonomically assigned to the genus Francsiella (e.g. using the software Kraken). For data analysis, the assembly of reads for the prediction of contigs is the first step. The assembled genomes should have a size of 1.7-1.9mB and an N50 value of 15kB. For high-resolution genotyping, methods based on predefined canonical single nucleotide (SNPs), such as with the CanSNPer tool are useful. In addition, methods based on core-genome MLST have become established. As an alternative to methods based on assembled genomes, methods that map reads to reference genomes and detect previously unknown SNPs (e.g. Snippy) have been established for high-resolution genotyping. An open-source bioinformatics pipeline performing QC, assembly, canonical SNP typing and whole-genome SNP typing is available at https://gitlab.com/FLI_Bioinfo/WGSBAC."

SHEVTSOV V, KAIRZHANOVA A, SHEVTSOV A, SHUSTOV A, KALENDAR R, ABDRAKHMANOV S, LUKHNOVA L, IZBANOVA U, RAMANKULOV Y, VERGNAUD G. Genetic diversity of *Francisella tularensis* subsp. *holarctica* in Kazakhstan. *PLoS Negl Trop Dis.* 2021 May 17;15(5):e0009419.

LINDE J, HOMEIER-BACHMANN T, DANGEL A, RIEHM JM, SUNDELL D, ÖHRMAN C, FORSMAN M, TOMASO H. Genotyping of *Francisella tularensis* subsp. *holarctica* from Hares in Germany. *Microorganisms.* 2020 Dec 5;8(12):1932

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KEVIN M, GIRAULT G, CASPAR Y, CHERFA MA, MENDY C, TOMASO H, GAVIER-WIDEN D, ESCUDERO R, MAURIN M, DURAND B, PONSART C, MADANI N. Phylogeography and Genetic Diversity of *Francisella tularensis* subsp. *holarctica* in France (1947-2018). *Front Microbiol*. 2020 Mar 4;11:287.

LINDGREN P, MYRTENNÄS K, FORSMAN M, JOHANSSON A, STENBERG P, NORDGAARD A, AHLINDER J. A likelihood ratio-based approach for improved source attribution in microbiological forensic investigations. *Forensic Sci Int*. 2019 Sep;302:109869.

BIRDSELL DN, YAGLOM H, RODRIGUEZ E, ENGELTHALER DM, MAURER M, GAITHER M, VINOCUR J, WEISS J, TERRIQUEZ J, KOMATSU K, ORMSBY ME, GEBHARDT M, SOLOMON C, NIENSTADT L, WILLIAMSON CHD, SAHL JW, KEIM PS, WAGNER DM. Phylogenetic

Analysis of *Francisella tularensis* Group A.II Isolates from 5 Patients with Tularemia, Arizona, USA, 2015-2017. *Emerg Infect Dis.* 2019 May;25(5):944-946.

AFSET JE, LARSSEN KW, BERGH K, LARKERYD A, SJODIN A, JOHANSSON A, FORSMAN M. Phylogeographical pattern of Francisella tularensis in a nationwide outbreak of tularaemia in Norway, 2011. *Euro Surveill.* 2015 May 14;20(19):9-14.

3.1.X. Mammalian tuberculosis (infection with *Mycobacterium tuberculosis* complex)

General comment:

The EU can in general support this new chapter.

In the Summary (LINE 29), Introduction (LINE 182) and Diagnostic Techniques (TABLE 1), the tests for camelids are mentioned. Unfortunately, camelids are not mentioned in later sections of the chapter. According to the SOPs from the EU-RL for bovine Tuberculosis (https://www.visavet.es/bovinetuberculosis/databases/bt-protocols.php), the combination of the intradermal tuberculin test and serology for detection of specific antibodies is recommended. Therefore, Section 2.2. should be expanded to include interpretation of the skin test results in camelids and the indication that the diagnostic procedure should be complemented by the use of serology. On the other hand, the serology Section 3.2. should include the diagnostic procedure for camelids as well as the indication that it is to be used as complementary test to the skin test as well as 15-30 days later repeatedly. The diagnostic procedure in camelids must be described in such specific terms because many countries impose restrictions on the international movement of these animals.

Furthermore, terminology for the New Zealand wildlife reservoir host, the brush-tailed possum (*Trichosurus vulpecula*) is inconsistent throughout the document.

Specific comments are provided below.

Specific comments:

LINES 28-29: "Delayed hypersensitivity test (tuberculin skin test): This test is the standard method for detection of tuberculosis in live cattle, small ruminants, deer, pigs and camelids, among other species." May merit specific comment regarding accuracy in Camelids.

LINE 48: Please insert "For this reason, vaccination of cattle is prohibited in many countries." after "... tuberculin-based immunological tests".

LINE 50: Please replace "licensed" and "", respectively, with "granted regulatory approval", as this is an agreed term that works in all countries. Similarly, please replace "marketing authorisation" with "regulatory approval" in **LINE 645**.

LINES 76-77: *M. microti* was occasionally reported in several species but it would be noteworthy to mention its high diffusion in wild boar:

Boniotti *et al*. 2014. Detection and molecular characterization of *Mycobacterium microti* isolates in wild boar from Northern Italy. J. Clin. Microbiol. 52: 2834-2843.

Chiari *et al*. 2016. Spatiotemporal and ecological patterns of *Mycobacterium microti* infection in wild boar. Transbound. Emerg. Dis. 63: e381-e388.

LINE 88: "...(primarily the brushtail opossum, *Trichosurus vulpecula)..." S*ome sources state a preference for using the term 'possum' for Australian/New Zealand species (Phalangeriformes) and 'opossum' for North American species (Didelphimorphia).

LINES 199-120: In wild boar lesions are mainly localised in head lymph nodes (rethropharyngeal and/or mandibular).

LINE 131: The word 'unattended' is rendered redundant by the use of the word 'untreated'.

LINE 133: The correct term is 'pathognomonic' not 'pathognomic'.

LINE 147: Suggest inserting "and echography" after "radiological examination".

LINE 156: Please insert "However, the microscopic test can often give negative results, even in sick animals, due to the limited presence of mycobacteria." after "central caseating necrosis".

LINES 329-333: The validation of Real Time PCR targeting the IS6110 published by EURL-TB could be mentioned here: Lorente-Leal *et al*. 2021. Direct PCR on tissue samples to detect Mycobacterium tuberculosis complex: an alternative to the bacteriological culture. J. Clin. Microbiol. 59:e01404-20. https:// doi.org/10.1128/JCM. 01404-20.

LINE 361-363: The sentence should be amended as follows:

"Currently a 24-loci MIRU-VNTR PCR assay is used for *Mycobacterium tuberculosis* (Supply et al., 2006) but optimisation of the combination of loci for a specific region is recommended for *M. bovis* and *M. caprae* to decrease the cost and time spent performing the assays whilst maximising discriminatory power.".

LINE 396: Please replace "measured" with "evaluated". Indeed, in pigs, the diameter of the reaction is not measured, but its character is evaluated.

LINE 405: The usual interval is 6 weeks.

LINES 422-423: Please replace the sentence "All measurements of skin thickness should be recorded in whole millimetres as measurements in fractions of millimetres may provide a false sense of precision." with "All skin thickness measurements should be recorded in millimeters to the first decimal place.".

LINES 461, 467, 478, 485, 503: The interval should be 6 weeks.

LINE 478: There is lack of consistency: ">2 and 4mm" in the table above and "≥2 and 4 mm" here. Also in the table below it is inconsistent.

LINES 463 – 471: This paragraph (in Section 2.2.2.1. Single Cervical Test) moves beyond interpretation of the test itself to its use in the context of control/eradication. No such similar paragraph is provided in respect of 2.2.2.2 the Comparative Cervical Test (CCT) or 2.2.2.3 Caudal Fold Test (CFT). For consistency, if included in respect of one test, it would be logical to provide a similar paragraph in respect of both other tests.

3.2.1. Acarapisosis of honey bees

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 10: Suggest to add at end of sentence 'and can also be found at the base of the bee's wings'.

LINES 19, 20, 71, 72, 85, 97, Table 1: Please replace "infection" with "infestation", as was correctly done in the title of the chapter and several times in the text.

LINE 23: The insertion 'up to 20 eggs' may need to be referenced as it was accepted that females laid approximately 8 eggs.

LINE 26: The term 'detected' rather than the term 'demonstrated' may be a better**.**

LINES 37-39: May be worth mentioning that this method is known as the 'Thoracic disc method'.

LINE 76: May be better to replace 'attack by A. woodi' with 'A. woodi infestation'.

LINE 85-87: Suggest adding 'Heavy mite infestations affect bee metabolism and the ability of colonies to regulate the cluster temperature (Skinner, 2000), hence chilling can be a significant cause of death.'

LINE 100: This is not referenced and may need for clarification. Note that drones are only in the colony during the Summer while infestation loads are highest in Winter/Early Spring. The winter cluster facilitates more reproductive cycles.

LINE 112: Suggest insert 'for up to 24 hours' after '(-20C)'.

LINE 113-114: Text is cumbersome and confusing.

LINES 356-357: There is an error in the reference, it should be "SCOTT-DUPREE" instead of "DUPREE", and the journal should be "Apidologie" instead of "J Apic Res".

LINE 400: In references section, please insert reference: Skinner, Alison (2000): Impacts of Tracheal Mites (Acarapis woodi (Renne)) on the respiration and Thermoregulation of Overwintering Hioney bees in a Temperate Climate. Masters Thesis, University of Guelph. Accession Number: 0-612-55716-2

3.3.9. Fowl cholera

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINES 376, 506: Please replace the title with “Requirements for regulatory approval”, as was done in other chapters adopted recently.

LINES 391: Please replace “licensure or registration” with “regulatory approval”, as this is an agreed term that works in all countries.

LINES 512: Please replace “licensing” with “regulatory approval”, as this is an agreed term that works in all countries.

3.3.15. Turkey rhinotracheitis (avian metapneumovirus)

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINES 119,120: Suggest further elaboration of co-infection, this could be achieved by insertion prior to sentence commencing with 'Morbidity can be as high as 100% ...' of the sentence 'In addition, other co-infections with viruses such as Infectious Laryngotracheitis, Infectious bronchitis, Paramyxovirus -1 (currently named Avian Orthoavulavirus-1) or fungi such as Aspergillus fumigatus (Croville *et al*. 2018) have been reported'.

LINE 164: Include in description of clinical signs 'swelling of infraorbital sinuses'.

LINE 676: Please replace the title with '2.3. Requirements for regulatory approval', as was done in other chapters adopted recently.

LINES 692, 693: Please replace 'licensing' with 'regulatory approval', as these are the agreed terms that work in all countries.

LINE 766: Insert reference CROVILLE G., FORET C., HEUILLARD P., SENET A., DELPONT M., MOUAHID M., DUCATEZ M., KICHOU F., GUERIN J. (2018). Disclosing respiratory co-infections: a broad-range panel assay for avian respiratory pathogens on a nanofluidic PCR platform, Avian Path. 47:3, 253-260.

3.6.2. Contagious equine metritis

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINES 131-132: Should this not be “oxidase and catalase positive”. In the next sentence we suggest “should” be replaced by the word “can” to allow confirmation with other confirmatory tests such as agglutination tests.

Section 1, Identification of the agent: There are subsections on real time PCR and sequencing methods. Should consideration be given to including MALDI-TOF?

LINES 285-286: “These may be used to enhance the testing capabilities of laboratories already using culture, but at present their validation is uncertain.” could be replaced by “These may be used to enhance the testing capabilities of laboratories already using culture if their performance has been previously validated as equivalent to that of the real-time PCR 1 (Wakeley *et al*., 2006) and/or the real-time PCR 2 (Nadin-Davies *et al*., 2015).”

LINES 317-320: “Published data indicate the organism can remain viable after freezing semen, and it can be readily recovered from fresh semen by culture, but its growth is inhibited by antibiotics in semen extender (Klein *et al*., 2012; Olivieri *et al*., 2011).” The end of this sentence is not always true as shown Delerue *et al*., 2019 data[2] where a strain of *T. equigenitalis* was isolated by culture method, and detected directly by the real-time PCR 1 with a Ct-value of 20.07, in frozen-thawed stallion semen cryopreserved over a six-year period; the extender used during the cryopreservation process contained amphotericin, gentamicin, and penicillin.

This sentence could be replaced by “Published data indicate the organism can remain viable after freezing semen, and it can be readily recovered from fresh semen by culture. Its growth could be inhibited by antibiotics in semen extender (Klein *et al*., 2012; Olivieri *et al*., 2011) but this is not always the case (Delerue *et al*., 2019).”

[2] Delerue M, Breuil MF, Duquesne F, Bayon-Auboyer MH, Amenna-Bernard N & Petry S (2019) Acute Endometritis due to *Taylorella equigenitalis* Transmission by Insemination of Cryopreserved Stallion Semen. J. Equine Vet. Sci. 78:10-13

PMID: 31203971

3.6.11. Glanders and melioidosis

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINES 151-152: "72-hour incubation of cultures is recommended; glycerol enrichment is particularly useful." should be replaced by "Incubation of cultures for 72 hours is recommended; glycerol enrichment is particularly useful."

LINES 381-382: "A commercially available C-ELISA has recently been developed using anti-s B. mallei LPS Mab" – this is competitive ELISA test developed by Lillidale which is no longer available. This paragraph needs to be updated with the recent new developments/validations.

LINES 392-393: "An immunoblot assay was developed for the serodiagnosis of glanders, but further validation was impossible because of the lack of a positive serum control panel (Katz *et al*., 1999)." should be replaced by "Immunoblot assays were developed for the serodiagnosis of glanders (Katz *et al*., 1999, Elschner *et al*., 2011)."

LINES 393-397: "Recently, the development of an immunoblot using *B. mallei* LPS antigen was reinitiated. The aim was to obtain a more sensitive test than the CFT in order to retest false positive CFT sera in non-endemic areas (Elschner *et al*., 2011). The developed assay is based on crude antigen preparations of the *B. mallei* strains Bogor, Zagreb and Mukteswar, which are also the basis of most CFT antigen formulations." should be replaced by "Recently, the further development of an immunoblot using *B. mallei* LPS antigen was reinitiated and the validation was performed in comparison to CFT and a commercial recombinant double antigen-based ELISA using 400 negative and 370 glanders positive field samples of equidae including horses, mules, and donkeys (Elschner *et al*., 2021). The immunoblot was significantly more specific (99.2%) than the CFT (97.0%). Considering the comparable sensitivities of CFT (96.5%) and immunoblot (97.3%), the immunoblot is a suitable confirmatory test. However, the test is preferably suitable for individual samples, is time consuming and restricted to few experienced laboratories. The assay is based on crude antigen preparations of the *B. mallei* strains Bogor, Mukteswar, and Bahrain1."

LINES 404-408: "171 sera of glanderous horses and mules from Pakistan and Brazil and 305 sera of negative German horses were investigated and all glanders positive and negative animals were diagnosed correctly, however the test has not been fully validated to date. This test is not able to differentiate glanders from melioidosis infection and it has not yet been evaluated for use in donkeys because of the lack of a significant number of positive control sera." Should be replaced by „This test is not able to differentiate glanders from melioidosis infection."

3.8.11. Scrapie

General comment:

The EU in general supports the revised chapter.

Please replace "normal prion protein" with "cellular prion protein" throughout the chapter.

Specific comments are provided below.

Specific comments:

LINES 23-24: "In sheep, it has also been reported in animals with PrP genotypes that are ~~relatively~~ resistant to classical scrapie whereas with regards to atypical scrapie, variations at some codons can affect susceptibility."

LINES 36-37: "The majority of cases occur between 2 and 5 years of age. Clinical disease develops only if the agent enters the CNS. Atypical scrapie, where it presents clinically, is reported mostly in older animals~~, and occurs with a geographical distribution suggestive of a spontaneous disease, although it has been transmitted experimentally.~~"

We suggest this amendment since the spontaneous character of the disease is speculative. However we agree with the statement in **LINE 22** that "The epidemiology is consistent with a non-contagious condition that occurs sporadically".

LINE 42: "and" instead of "or", in many cases HE alone is not sufficient to diagnosis a scrapie case.

LINES 42-43: "Immuno-detection of PrPSc in brainstem samples forms the basis of the rapid tests, which are most often used in active surveillance programmes."

LINE 49: Please include the most important tissue samples for biopsies: "using biopsied tissue, e.g. from palatine tonsil and/or Rectoanal mucosa-associated lymphoid tissue (RAMALT)"

LINES 49-51: "It is not, however, appropriate for detection of atypical scrapie as there is little or no involvement of the lymphoreticular system, or of a proportion of classical scrapie cases, so it can be used only to confirm the presence of infection and cannot be used to prove its absence."

LINE 72: Please add the following reference after "conditions": EFSA (European Food Safety Authority), Arnold M, Ru G, Simmons M, Vidal-Diez A, Ortiz-Pelaez A and Stella P, 2021. Scientific report on the analysis of the 2-year compulsory intensified monitoring of atypical scrapie. EFSA Journal 2021;19(7):6686, 45 pp. https://doi.org/10.2903/j.efsa.2021.6686

LINE 120: Please consider adding a table with grouping of alleles by level of susceptibility with explicit indication of the resistance associated to ARR allele.

LINES 125-126: "Very little is known about the influence of genetics on atypical scrapie in goats: one case–control study (Colussi *et al*., 2008) has shown an association between histidine at codon 154 and the disease (See EFSA [20211~~7~~] for recent overview)."

With the following references:

- Colussi, S., Vaccari, G., Maurella, C., Bona, C., Lorenzetti, R., Troiano, P., ... & Acutis, P. L. (2008). Histidine at codon 154 of the prion protein gene is a risk factor for Nor98 scrapie in goats. Journal of General Virology, 89(12), 3173-3176.
- EFSA (European Food Safety Authority), Arnold M, Ru G, Simmons M, Vidal-Diez A, Ortiz-Pelaez A and Stella P, 2021. Scientific report on the analysis of the 2-year compulsory intensified monitoring of atypical scrapie. EFSA Journal 2021;19(7):6686, 45 pp. https://doi.org/10.2903/j.efsa.2021.6686

LINE 131, section strain characterization: this is a very rudimentary description of scrapie strains focusing solely on ovine/caprine BSE. This should be amended to reflect the biodiversity of Classical scrapie strains properly.

For more information see EFSA Journal 2015;13(8):4197: “A spectrum of strains is responsible for Classical scrapie in sheep, and there may be variability in properties that affect the ability to cross the species barrier. There is experimental evidence that some isolates may not be completely stable, and their fundamental properties may shift on transmission. There is also potential heterogeneity of geographical distribution of individual strains. Evidence derived from a limited number of classical isolates cannot be extrapolated to represent the whole biological variability of Classical scrapie. In contrast to Classical scrapie, no strain variability has been observed so far among isolates of Atypical scrapie.”

Additionally, Nonno *et al*. (2020) concluded that “most field prion isolates showed composite strain features, with discrete strain components or sub-strains being present in different proportions in individual goats or tissues. This has important implications for understanding the nature and evolution of scrapie strains and their transmissibility to other species, including humans.”Line 147: Include goats as well “Most breed of sheep and goats”

LINE 189: “performed on the medulla oblongata and cerebellum”.

LINE 267: Include “neutral-buffered saline”, there are several fixations in pathology, this should be clarified right from the beginning : “or is fixed in neutral-buffered formalin for histological preparations.”

LINES 350-351: “In classical scrapie lesions are usually most apparent in the brainstem and frequently affect the dorsal nucleus of the vagus nerve and in the spinal tract nucleus of the trigeminal nerve.”

LINE 477: *“*Genetic screening for ~~resistance~~ the disease control”.

LINES 246-247: Please indicate where the EURL Discriminatory Testing Handbook is available.

LINE 495: “with genetic resistance to classical scrapie are”.

LINE 498: “allow selection for genetic resistance to classical scrapie in goats”.

LINES 496-498: “Consequently application of scrapie control and elimination programmes in this species has been successful only in limited cases were appropriate polymorphisms have been identified to allow selection for genetic resistance to scrapie in goats (EFSA, 2017; Georgiadou *et al*. 2017). While the possibility of applying a breeding programme for resistance in goats may not be feasible due to the low abundance of resistant polymorphisms, screening

programmes at local level can help to identify resistant goats to restock affected herds after the application of genetically-based selective culling, as it has been applied for decades in sheep."

3.8.13. Theileriosis in sheep and goats (infection with *Theileria lestoquardi*, *T. luwenshuni* and *T. uilenbergi*)

General comment:

The EU supports the revised chapter.

Specific comments:

None

3.9.3. Classical swine fever (infection with classical swine fever virus)

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINES 57-59: Please note that the only subunit vaccine based on the E2 protein ("Porcilis pesti", MSD) is no longer commercially available in the EU. The marketing authorisation has been withdrawn at the request of the marketing authorisation holder (https://www.ema.europa.eu/en/medicines/veterinary/EPAR/porcilis-pesti).

LINE 114: In **TABLE 1**, please replace "PCR" with "RT-PCR".

LINE 117: Please amend as follows: "RT-PCR = reverse-transcription polymerase chain reaction" (reference is made to section 1.1.4., Line 171).

LINE 716: Please replace "authorised" with "granted regulatory approval" as the term "authorised" is used only in some OIE member countries whereas the term "regulatory approval" works globally and has been used in other recently adopted Manual chapters. Furthermore, in **LINES 57 and 730,** please replace respectively "registered" and "licensed" with "approved" (same rationale as above).

LINE 822: Please replace "registration" with "regulatory approval" (as in **LINE 828**).

3.10.1. Bunyaviral diseases of animals (excluding RVF fever and Crimean–Congo haemorrhagic fever)

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 82: 2002 is not “recent”, therefore please delete that sentence and reference and replace with the following:

“A recent seroprevalence of CVV in sheep and livestock (cattle 20%, 33.3% goats, horses 69% and mule deer 50.8%) was conducted in Canada (Uehlinger *et al*. 2018 The Canadian Veterinary J. 5984) 413, 2018).”

LINE 86: Similarly, a more recent reference is provided below:

“In sheep five outbreaks of birth defects following asymptomatic dam CVV infection during the first 50 days of pregnancy, were reported”. See Waddell *et al*. 2019 Zoonoses and public health 66 (7) 739-758, 2019.

LINE 93: Please note that six human cases are reviewed in Waddell *et al*. 2019 Zoonoses and public health 66 (7) 739-758, 2019.

LINE 197: Please insert “and the agent was transmitted transovarially in the tick” after “(Daubney & Hudson, 1934)”.

3.10.2. Cryptosporidiosis

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 9: Please consider “diarrhoea” instead of “scour”.

LINE 66: Table 1, there are empty field in “Usual sites of infection”. Please fill.

LINE 66: Table, Confirm or correct “Ventricuus”.

LINE 84: Consider “diarrhoea” instead of “scouring”.

LINES 303, 312, 318: Is it possible to provide a maximum storage time?

LINE 329: This is not detailed enough. What is a “minimum volume”?

LINE 502: Please add “(IC)”

LINE 530: Is 5% $K_2Cr_2O_7$ really the only recommended preservative? Please consider to mention less toxic alternatives.

3.10.6. Mange

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 167: Although not common, cats should be mentioned among domestic hosts for *Sarcoptes scabei*.

LINE 278: Horses should be included. As mentioned on **LINE 270**, horses are frequently infected with Chorioptic mange.

LINE 446-449: Suggest preface the sentence beginning with "Transfer between hosts [...]" with "With one exception, *Demodex gatoi* which can be transferred between cats of any age," (Saari, S.A. 2009).

LINES 584-594: Suggest moving Section 3 "Serological test" up to "Detecting the agent", 1.3 Serological tests, starting on line 120.

LINE 651: Please insert this reference: Saari, S.A., Juuti, K.H., Palojärvi, J.H. *et al*. Demodex gatoi -associated contagious pruritic dermatosis in cats - a report from six households in Finland. Acta Vet Scand 51, 40 (2009).

EU COMMENTS on the proposed changes to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

3.10.7. Salmonellosis

General comment:

The EU can in general support this revised chapter. Specific comments are provided below.

Specific comments:

LINE 58: Why has the reference to Grimont and Weill (2007) been deleted? It looks as if the information about the Salmonella species and subspecies was taken over from this reference? Therefore please retain the reference to Grimont and Weill, 2007.

LINE 64: Add the reference to Grimont and Weill, 2007 after 'the White Kauffmann Le Minor scheme'.

LINE 85: According to Grimont and Weill, 2007, Pullorum is considered as a biovar of serovar Gallinarum and no longer as a serovar. Proposal for amendment: '... infections caused by S. Gallinarum, biovar Pullorum and S. Gallinarum, biovar Gallinarum, respectively.'

LINE 175: The culture media should also support growth in the presence of both matrix and competing flora. Please add 'and' at: '… of a relevant sample matrix and/or competing flora.'

LINE 182: Please add 'agar' after 'The modified semi-solid Rappaport Vassiliadis (MSRV) agar ….'

LINES 182-183: MSRV has been validated for environmental samples as well. Therefore, please retain environmental samples in the list as well. Proposal for amendment: '… has been validated for faecal, food, feed and environmental samples ….'.

LINES 187, 189: Please add 'agar' after MSRV.

LINES 191-193: This information seems to refer specifically to the use of MSRV agar as RVS broth and MKTTn broth are also effective for the detection of non-motile salmonellae. Proposal for amendment: '~~This method~~MSRV agar has been shown to be highly effective for detection of motile salmonellae.'

LINES 211, 217: Please change 'RSV' into 'RVS'.

LINES 217, 218: Please add 'agar' after MSRV.

LINES 257-259: Proposal for amendment: 'A wide range of chromogenic agars are ~~now~~ also available for the selective isolation of salmonellae.'.

LINE 270: Proposal for amendment: '… method for S. Gallinarum (biovars Gallinarum and Pullorum) in …'.

LINE 272: Proposal for amendment to bring in line with ISO 6579-1: 'Inoculate 15-20 ml MSRV agar in a Petri dish (diameter 90 mm) with …'.

LINES 314-315: The reference to the ISO document is not correct. Proposal for amendment: 'A miniaturised MPN method has been described by ISO in ISO/TS 6579-2:2012.'

LINE 334: The reference to the ISO document is not correct, please amend into: 'ISO/TR 6579-3:2014'.

LINES 336-337: Phagetyping is hardly ever performed anymore and may therefore be better to be deleted here. Proposal for amendment: '…to confirm the identity of an isolate, ~~including~~ or to conduct ~~phage typing, if serovar-specific typing phages are available, and for~~ genetic characterisation.'

LINE 361: Change 'accreditation body' into 'certified organisation'.

LINE 387: Add the year of publication of the new ISO standard: ISO 16140-6:2019 and place 'sub' (of subtyping) between brackets, thus '… validation of (sub)typing methods …'.

LINES 395-396: Proposal for amendment: '… infected with S. Gallinarum (biovars Gallinarum and Pullorum; see Chapter 3.3.11).'

LINE 449: Proposal for amendment: '… but in the case of S. Gallinarum (biovars Gallinarum and Pullorum), combined …'.

LINE 457: Change 'S. Pullorum' into 'S. Gallinarum'.

LINES 496, 510, 515-516: To be in line with the earlier described incubation temperature for the culture method for detection of Salmonella (as described in ISO 6579-1) it may be better to change '37 °C (±2 °C)' into '34-38 °C'.

LINES 634, 636: Please replace 'licensing' with 'regulatory approval', and 'licensed' with 'approved', respectively, as these are the agreed terms that work in all countries.

LINE 743: Please replace the title with '2.3. Requirements for regulatory approval', as was done in other chapters adopted recently.

LINES 858-859: Please retain the reference to Grimont and Weill (2007).

LINES 877-879: Please retain the reference to ISO/TR 6579-3:2014.